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Quantification of the spread of *Mycoplasma hyopneumoniae* in nursery pigs and effect of virulence difference between isolates

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Introduction

Although it is known that, in many herds, piglets can infect each other with *M. hyopneumoniae* during the nursery period (Clark et al., 1991, Vicca et al., 2002; Ruiz et al., 2003), quantification of the infection rate has not yet been performed for *M. hyopneumoniae*. The present study was conducted to quantify the transmission of *M. hyopneumoniae* under experimental conditions among weaned piglets, by calculating an adjusted reproduction ratio (R_n). Since previous work has clearly shown differences in virulence between *M. hyopneumoniae* isolates (Vicca et al., 2003), possible differences in transmission between groups infected with a high virulent and a low virulent *M. hyopneumoniae* isolate were also investigated.

Materials and methods

Experimental design and infection parameters

Forty-eight, 3 week-old weaned piglets that were free of *M. hyopneumoniae* and PRRSV were randomly assigned into 2 groups (3 pens in each group). At 28 days of age, within each group, 6 pigs (2 per pen) were randomly selected for inoculation with either a high or a low virulent *M. hyopneumoniae* isolate (seeder pigs). The inoculation day was designated as day 0 post-infection (0 DPI). At 2 DPI, 2 inoculated and 6 susceptible contact-animals were brought together in a pen (6 pens in total). The disease spread was monitored during a period of 6 weeks, corresponding with the duration of the nursery period mostly applied under field conditions in Western Europe.

Two Belgian *M. hyopneumoniae* field isolates, obtained from the lungs of pigs from 2 different Belgian pig herds, were used. These isolates had been determined to be high and low virulent during comparative studies (Vicca et al., 2003). The high virulent isolate was obtained from a herd experiencing clinical symptoms associated with enzootic pneumonia. The low virulent isolate was obtained from an infected herd without clinical symptoms (Vicca et al., 2002). Twelve pigs were inoculated intratracheally with 7 ml inoculum, containing 1×10^7 CCU/ml of either the high or low virulent *M. hyopneumoniae* isolate.

Each group of pigs was observed daily for 15 min for presence of respiratory disease. A clinical respiratory disease score (RDS) that could range from 0 to 6 was assessed daily (Halbur et al., 1996). Summation of coughing scores was made and a median respiratory score was calculated for the 42 days of the trial.

Blood samples from all pigs were taken upon arrival, at 23, 32 and 43 DPI to detect antibodies against *M. hyopneumoniae* using the DAKO[(r)] Mh ELISA (Feld et al., 1992).

Macroscopic and typical *M. hyopneumoniae* induced pneumonic lesions were quantified using a lung lesion score diagram (Hannan et al., 1982). Total lung scores could vary between score 0 (no lesions) and a theoretical maximum of 35.

All piglets were euthanized at 43 DPI. The right lung was flushed with 50 ml sterile phosphate buffered saline (PBS) and the obtained BAL fluid was investigated with a nPCR-test (Stärk et al., 1998). If the test was positive, the pig was considered as being infected because nPCR is the most sensitive parameter to assess *M. hyopneumoniae* infection at individual level (Kurth et al., 2002).

Statistical analyses

The transmission of *M. hyopneumoniae* in each group was estimated using a stochastic infection model. It was assumed that the process of transmission of *M. hyopneumoniae* among the piglets was in accordance with the Susceptible-Infectious (S-I) model. The number of contact infections, determined by the number of nPCR positive BAL fluids at the end of the trial, was the observed variable (X_i, also called the 'final size' of the outbreak). The final size distribution depends on the reproduction ratio (R_n-value) (Diekmann et al., 1990), and can be defined as the mean number of secondary cases caused by one typical infectious pig during the observation period. There were 6 populations with N = 8 animals, where initially 2 animals were infectious ($I_0 = 2$) and 6 animals were susceptible ($S_0 = 6$) (non-infected contact-exposed pigs). The probability distribution of the final size was represented by F ($X_i \mid R_n$, N, S_0, I_0).

A linear mixed effect model, using pen as random variable, was used for the analysis of clinical symptoms. Lung lesion scores were log transformed and analyzed using logistic regression analysis. Time until first seroconversion

was analyzed by Cox regression survival analysis. Results of inoculated pigs and contact pigs were always analyzed separately.

Results

Major parameters (nPCR on BAL and R_n value)

All experimentally infected animals were positive for *M. hyopneumoniae* in the BAL fluid by nPCR-testing at 43 DPI. In the high virulent group, 10 out of 18 (56%) contact piglets were found positive for *M. hyopneumoniae*, while in the low virulent group, 6 out of 18 (33%) contact animals were found positive for *M. hyopneumoniae*. The distribution in each pen of contact piglets that became contact-infected is shown in **Table 1**.

The R_n-value (95% CI) was 1.47 (0.68 - 5.38) and 0.85 (0.33 - 3.39) for animals infected with the high virulent and the low virulent isolate, respectively. Since no significant difference was found between the R_n-value in these two groups (p = 0.53), the overall R_n-value was calculated, by combining the outcomes of the six transmission experiments. This overall R_n-value was 1.16 (0.94 - 4.08).

Minor parameters (clinical symptoms, serology, lung lesions)

Piglets inoculated with the high virulent isolate had a first positive coughing score at 6.83 DPI, while animals inoculated with the low virulent isolate had the first coughing score only at 10.83 DPI. First positive coughing score was noted on average at 14.56 DPI and at 18.89 DPI, for contact pigs in the high and low virulent group, respectively. The median respiratory score for the inoculated animals was 85.0 and 34.0 in the high virulent and in the low virulent groups, respectively (p < 0.05). The median respiratory score for the contact pigs was 16.0 and 8.0 in the high virulent and in the low virulent groups, respectively (p > 0.05).

Upon arrival, all piglets were serologically negative for *M. hyopneumoniae*. In the high virulent group, the number of inoculated pigs (6) that was serologically positive was 4, 5 and 5 at 23, 32 and 43 DPI, respectively. Only 2 out of 18 contact pigs were serologically positive at 43 DPI. In the low virulent group, the number of inoculated pigs (6) that was serologically positive was 0, 1 and 4 at 23, 32 and 43 DPI, respectively. None of the contact pigs was serologically positive. Although the high virulent isolates induced an earlier seroconversion compared with the low virulent isolates, the time until first seroconversion was not significantly different between both inoculated groups (p=0.147).

The mean lung lesion scores of the inoculated pigs were 8.50 and 1.93 for animals experimentally infected with a high virulent and a low virulent isolate, respectively (p > 0.05). The mean lung lesion scores of the contact animals were 0.61 and 0.18 in the high virulent and low virulent groups, respectively (p >0.05).

Discussion

According to what was expected based on the results of previous experiments (Etheridge et al., 1979) and based on data from field observations (Vicca et al., 2002), transmission of *M. hyopneumoniae* during the 6 weeks observation period could be demonstrated in all experimental groups. In addition to comparing transmission rates of different isolates of a pathogen or different pathogens, the calculation of reproduction ratios is also interesting to assess the influence of different control strategies (e.g. vaccination or medication) on the spread of pathogens. An R_0 value was not calculated because the exact duration of the infectious period for *M. hyopneumoniae* cannot be established reliably in the live animal at this moment. Therefore, an adjusted reproduction ratio (R_n) was calculated and a specified period of 6 weeks corresponding with the nursery period was taken as the infectious period. The nursery period is particularly important because a few animals, which become infected before weaning, can infect a considerable number of other piglets during this period. In this way, several piglets become infected with *M. hyopneumoniae* before the finishing period, which might result in long-lasting and considerable economic losses attributed to mycoplasmal pneumonia (Maes et al., 2003). The overall R_n-value of 1.16 indicated that the spread of *M. hyopneumoniae* was not excessive, but that in general, the infection will be maintained and will even result in at least a doubling of the infectious animals at the end of the nursery period. Based on positive findings of *M. hyopneumoniae* in sows up to the seventh parity (Calsamiglia and Pijoan, 2000), it is reasonable to expect that fattening pigs infected with *M.*

Table 1: Distribution of infection (43 days post infection) in 6 different nursery pens measured by nPCR in bronchoalveolar lavage (BAL) fluid in a study of transmission of *M. hyopneumoniae*. Table shows # of infected animals / # of susceptible animals.

High virulent *M. hyopneumoniae*			Low virulent *M. hyopneumoniae*		
Pen 1	**Pen 2**	**Pen 3**	**Pen 4**	**Pen 5**	**Pen 6**
2/6	3/6	5/6	2/6	2/6	2/6

hyopneumoniae will remain infected during the rest of the fattening period, and might infect other animals.

The difference in transmission between the high virulent and the low virulent isolate was not statistically significant, but there was a tendency towards a more intensive spread in the high virulent group. The reason for the difference in spread is not clear at this moment but it might be due, among other factors, to a higher load of mycoplasmas in the respiratory tract of these animals. The present study confirmed results of previous experiments (Vicca et al., 2003) concerning the difference of the high virulent and the low virulent strains, but the number of inoculated animals was too limited to find significant results for all parameters.

The results of the present study may not be extrapolated as such to field conditions for a number of reasons. The inoculated animals became fully infectious only after one or two weeks (Sörensen et al., 1997) of the experiment because they were infected as late as two days before the start of the experimental period. In field situations, piglets can become infected already at a younger age. In addition, other respiratory infections are usually more prevalent under field conditions and the stocking density in the pens is mostly higher. On the other hand, the seeder pigs in the present study received a high dose of *M. hyopneumoniae* organisms whereas under field conditions, the infection dose might be lower.

In conclusion, under the present experimental conditions, the transmission of *M. hyopneumoniae*, assessed for the first time by a reproduction ratio, showed that one piglet infected before weaning will infect at least one penmate during the nursery phase.

Acknowledgements

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